Patent- og Varemærkestyrelsen
Helgeshøj Allé 81
2630 Tåstrup

20-JUN-2000

Reference	Country
Agent 29. JUN. 2000	Short title
Action	Term —

SLK 000629 SLK

Deres ref: PA 2000 00991
Vor ref: 10057.000-DK, SLK/SURH

Novo Nordisk

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Enzyme Business
Patents

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A/S Reg. No. 16201

Dansk patentansøgning Nr. PA 2000 00991

Vi skal herved meddele Dem, at vi ønsker at tilbagetage ovennævnte ansøgning, som blev indleveret den 26-JUN-2000.

De bedes venligst bekræfte, at tilbagetagelsen har fundet sted ved at returnere vedlagte kopi af dette brev i underskrevet stand.

Med venlig hilsen
Enzyme Business Patents

Sten Lottrup Knudsen

Dato: ______________ Modtaget af: ______________________

Modtaget
28 JUNI 2000
Patent- og Varemærkestyrelsen

Cc: Journal

183 106

NZAS-0013102

19

Patent- og Varemærkestyrelsen
Helgeshøj Allé 81
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28-JUN-2000

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Med venlig hilsen
Enzyme Business Patents

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Dato: ________________ Modtaget af: ______________________________

Cc: Journal

183 106

NZAS-0013103

FRIST ×2

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Reference	Country
Agent SLK 00 06 27 27. JUN. 2000 SLK	Short title
Action	Term Je10 00 06 27

NOTERET

Je10 00 06 27

Vores ref: PA 2000 00991/DOK2/SPI
Deres ref: 10057.000-DK

Dato: 26. juni 2000

Novo Nordisk A/S

Vedrørende ansøgning om Field of the Invention .

De har den 26. juni 2000 indleveret en ansøgning om patent.

Vi har givet ansøgningen nr. PA 2000 00991. Vi beder Dem om at oplyse dette nummer ved henvendelse til os om denne sag.

Hvis De ikke har indbetalt ansøgningsgebyret endnu, vil vi senere sende et girokort på de gebyrer, som skal betales for behandlingen af ansøgningen. Vi gør Dem opmærksom på, at disse gebyrer ikke er momspligtige.

Med venlig hilsen

Patent- og Varemærkestyrelsen

NZAS-0013104

Patentansøgning

Patent- og Varemærkestyrelsen
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Læs venligst vejledningen til de enkelte punkter

2. Ansøgers fuldmægtigs referencenr.
10057.000-DK

3. International indleveringsdag: ☐ Kapitel I
Internationalt ansøgningsnr.: ☐ Kapitel II

4. Ansøger (fulde navn og adresse): ☐ Flere ansøgere på bagsiden
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5. ~~Fuldmægtig (navn og adresse)~~: Brev- og fakturamodtager

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Telefon: Telefax:

6. Opfinder (fornavn, efternavn, adresse):

6a. Opfinder (fornavn, efternavn, adresse): ☐ Flere opfindere på bagsiden

7. Opfindelsens benævnelse:

8. Prioritetspåstand(e): ☐ Flere prioritetspåstande på bagsiden

Dato	Land	Nr.
Dato	Land	Nr.
Dato	Land	Nr.

9. ☒ Ansøgningen omfatter deponering af en prøve af biologisk materiale, som angivet i patentlovens § 8a, stk. 1.

10. ☒ Ansøgningen omfatter en sekvensliste.

11. ☐ Ansøgningen er fremkommet ved deling eller udskillelse.
Stamansøgningens nr.: Ansøgt løbedag:

13. ☐ Ansøgningen er tidligere indleveret pr. telefax den:

14. Dato og underskrift: 26-06-2000

Sten Lottrup Knudsen
Novo Nordisk A/S

1. Gebyrer:
- ☐ Ansøgningsgebyr
- ☐ Kravgebyr
- ☐ ITS-nyhedsundersøgelsesgebyr

12. Bilagsfortegnelse:
- ☒ fremmedsproget beskrivelse
- ☐ dansk beskrivelse i 1 ekspl.
- ☐ sammendrag i 1 ekspl.
- ☐ tegninger i 1 ekspl.
- ☐ prioritetsdokument
- ☐ fuldmagt
- ☐ overdragelsesdokument
- ☐
- ☐

Fig nr. ________ ønskes publiceret sammen med sammendraget.

15. Behandling af fremmedsproget ansøgning mm. ønskes
- ☐ norsk
- ☐ svensk
- ☐ engelsk

1.1-jan00/ls

NZAS-0013105

LIPOLYTIC ENZYME

FIELD OF THE INVENTION

The present invention relates to a nucleic acid sequence encoding a lipolytic enzyme from *Fusarium culmorum*, as well as a recombinant method of producing the
5 lipolytic enzyme.

BACKGROUND OF THE INVENTION

Lipolytic enzymes (such as lipases and phospholipases) are known to be useful, e.g., in baking and detergents. A lipolytic enzyme from *Fusarium culmorum* CBS 513.94 and its N-terminal sequence are disclosed in US 5830736. A li-
10 pase/phospholipase from *Fusarium oxysporum* and its sequence are disclosed in WO 98/26057.

The enzyme yield of the wild-type strain is very low, and recombinant productions promises to be an economical way of producing the enzyme.

SUMMARY OF THE INVENTION

15 The inventors have isolated a gene encoding a lipolytic enzyme from *Fusarium culmorum* CBS 513.94 and cloned it into an *E. coli* strain. Accordingly, the invention provides a DNA sequence encoding a lipolytic enzyme.

The nucleic acid sequence of the invention may comprise a nucleic acid sequence which encodes a lipolytic enzyme and comprises:

20 a) the DNA sequence encoding a mature lipolytic enzyme cloned into a plasmid present in *Escherichia coli* DSM 13537,

b) the DNA sequence encoding a mature lipolytic enzyme shown in SEQ ID NO: 1, or

c) an analogue of the DNA sequence defined in a) or b) which

25 i) has at least 80 % homology with said DNA sequence, or

ii) hybridizes at high stringency with said DNA sequence, its complementary strand or a subsequence thereof.

Other aspects of the invention provide a recombinant expression vector comprising the DNA sequence, and a cell transformed with the DNA sequence or the
30 recombinant expression vector. The invention also provides a recombinant methods of producing the lipolytic enzyme.

A comparison with full-length prior-art sequences shows that the mature amino acid sequence of the lipolytic enzyme from *Fusarium culmorum* has 84 % ho-

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mology with the lipase/phospholipase from *Fusarium oxysporum* described above, and the corresponding DNA sequences show 79 % homology.

DETAILED DESCRIPTION OF THE INVENTION

Genomic DNA source

5 The DNA sequence of the invention may be derived of *Escherichia coli* DSM 13537 which contains a gene encoding the lipolytic enzyme.

E. coli DSM 15357 was deposited by the inventors on 15 June 2000 under the terms of the Budapest Treaty with the DSMZ - Deutshe Sammlung von Microorganismen und Zellkulturen GmbH, Mascheroder Weg 1b, D-38124 Braunschweig. 10 DE, Germany.

Lipolytic enzyme

The lipolytic enzyme encoded by the DNA sequence of the invention is able to hydrolyze carboxylic ester bonds and is classified as EC 3.1.1 according to Enzyme Nomenclature 1992, Academic Press, Inc. The enzyme has lipase (triacylglyc-15 erol lipase) activity (EC 3.1.1.3) and may also have phospholipase activity.

Further properties of the lipolytic enzyme are described in US 5830736.

Recombinant expression vector

The expression vector of the invention typically includes control sequences encoding a promoter, operator, ribosome binding site, translation initiation signal, 20 and, optionally, a selectable marker, a transcription terminator, a repressor gene or various activator genes. The vector may be an autonomously replicating vector, or it may be integrated into the host cell genome.

Production by cultivation of transformant

The lipolytic enzyme of the invention may be produced by transforming a 25 suitable host cell with a DNA sequence encoding the lipolytic enzyme, cultivating the transformed organism under conditions permitting the production of the enzyme, and recovering the enzyme from the culture.

The host organism is preferably a eukaryotic cell, in particular a fungal cell, such as a yeast cell or a filamentous fungal cell, e.g. a strain of *Aspergillus*, 30 *Fusarium*, *Trichoderma* or *Saccharomyces*, particularly *A. niger*, *A. oryzae*, *F. graminearum*, *F. sambucinum*, *F. cerealis* or *S. cerevisiae*. The production of the lipolytic enzyme in such host organisms may be done by the general methods

described in EP 238,023 (Novo Nordisk), WO 96/00787 (Novo Nordisk) or EP 244,234 (Alko).

Hybridization

The hybridization is used to indicate that a given DNA sequence is analo-
5 gous to a nucleotide probe corresponding to a DNA sequence of the invention. The hybridization conditions are described in detail below.

Suitable conditions for determining hybridization between a nucleotide probe and a homologous DNA or RNA sequence involves presoaking of the filter containing the DNA fragments or RNA to hybridize in 5 x SSC (standard saline citrate) for
10 10 min, and prehybridization of the filter in a solution of 5 x SSC (Sambrook et al. 1989), 5 x Denhardt's solution (Sambrook et al. 1989), 0.5 % SDS and 100 μg/ml of denatured sonicated salmon sperm DNA (Sambrook et al. 1989), followed by hybridization in the same solution containing a random-primed (Feinberg, A. P. and Vogelstein, B. (1983) *Anal. Biochem.* 132:6-13), ^{32}P-dCTP-labeled (specific activity >
15 1 x 10^9 cpm/μg) probe for 12 hours at approx. 45°C. The filter is then washed two times for 30 minutes in 2 x SSC, 0.5 % SDS at a temperature of at least 55°C, more preferably at least 60°C, more preferably at least 65°C, even more preferably at least 70°C, especially at least 75°C.

Molecules to which the oligonucleotide probe hybridizes under these condi-
20 tions are detected using an x-ray film.

Alignment and homology

The lipolytic enzyme and the nucleotide sequence of the invention may have homologies to the disclosed sequences of at least 85 %, particularly at least 90 % or at least 95 %, e.g. at least 98 %.

25 For purposes of the present invention, alignments of sequences and calculation of homology scores were done using a Needleman-Wunsch alignment (i.e. global alignment), useful for both protein and DNA alignments. The default scoring matrices BLOSUM50 and the identity matrix are used for protein and DNA alignments respectively. The penalty for the first residue in a gap is -12 for proteins and -
30 16 for DNA, while the penalty for additional residues in a gap is -2 for proteins and -4 for DNA. Alignment is from the FASTA package version v20u6 (W. R. Pearson and D. J. Lipman (1988), "Improved Tools for Biological Sequence Analysis", PNAS 85:2444-2448, and W. R. Pearson (1990) "Rapid and Sensitive Sequence Comparison with FASTP and FASTA", Methods in Enzymology, 183:63-98).

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Lipase activity (LU)

A substrate for lipase is prepared an emulsion of 5 % by volume of tributyrin (glycerin tributyrate) using 0.1 % gum Arabic as emulsifier. The hydrolysis of tributyrin at 30 °C at pH 7 is followed in a pH-stat titration experiment. One unit of lipase 5 activity (1 LU) equals the amount of enzyme capable of releasing 1 µmol butyric acid/min at the standard conditions. 1 KLU = 1000 LU.

Use of lipolytic enzyme

The lipolytic enzyme of the invention can be used in various industrial application of lipolytic enzymes, e.g. in baking, detergents, diglyceride synthesis (EP 10 307154), acidolysis, interesterification (WO 8802775), ester hydrolysis, oil degumming (JP-A 2-153997, US 5264367), production of lysolecithin (JP patent 2794574, JP-B 6-087751) and in the process described in PCT/DK 00/00109.

Use in baking

The lipolytic enzyme of the invention can be used in the preparation of 15 dough, bread and cakes, e.g. to improve the elasticity of the bread or cake. Thus, the lipolytic enzyme can be used in a process for making bread, comprising adding the lipolytic enzyme to the ingredients of a dough, kneading the dough and baking the dough to make the bread. This can be done in analogy with WO 9404035 and EP 585988.

20 Use in detergent

The variant may be used as a detergent additive, e.g. at a concentration (expressed as pure enzyme protein) of 0.001-10 (e.g. 0.01-1) mg per gram of detergent or 0.001-100 (e.g. 0.01-10) mg per liter of wash liquor.

The detergent composition of the invention may for example be formulated 25 as a hand or machine laundry detergent composition including a laundry additive composition suitable for pre-treatment of stained fabrics and a rinse added fabric softener composition, or be formulated as a detergent composition for use in general household hard surface cleaning operations. In a laundry detergent, the variant may be effective for the removal of fatty stains, for whiteness maintenance and for dingy 30 cleanup. A laundry detergent composition may be formulated as described in WO 97/04079, WO 97/07202, WO 97/41212, PCT/DK WO 98/08939 and WO 97/43375.

The detergent composition of the invention may particularly be formulated for hand or machine dishwashing operations. e.g. as described in GB 2,247,025 (Unilever) or WO 99/01531 (Procter & Gamble). In a dishwashing composition, the

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variant may be effective for removal of greasy/oily stains, for prevention of the staining /discoloration of the dishware and plastic components of the dishwasher by highly colored components and the avoidance of lime soap deposits on the dishware.

5 **MATERIALS AND METHODS**

Methods

Unless otherwise stated, DNA manipulations and transformations were performed using standard methods of molecular biology as described in Sambrook et al. (1989) Molecular cloning: A laboratory manual, Cold Spring Harbor lab., Cold Spring 10 Harbor, NY; Ausubel, F. M. et al. (eds.) "Current protocols in Molecular Biology", John Wiley and Sons, 1995; Harwood, C. R., and Cutting, S. M. (eds.) "Molecular Biological Methods for Bacillus". John Wiley and Sons, 1990.

Enzymes

Enzymes for DNA manipulations (*e.g.* restriction endonucleases, ligases 15 etc.) are obtainable from New England Biolabs, Inc. and were used according to the manufacturer's instructions.

Plasmids/vectors

pT7Blue (Invitrogen, Netherlands)

Cloning

20 LA PCR™ in vitro Cloning Kit (TaKaRa) was used for cloning and was used according to the manufacturer's instructions.

Microbial strains

E. coli JM109 (TOYOBO, Japan)

E. coli JM110 (Invitrogen)

25 E.coli DB6507 (F,pnrF74::Tn5,seupE44, lacY1, ara-14,galK2, xyl-5, mtl-1, leuB6, proA2, hsdS20, recA13, rpsL20, thi-1, lambda-)

A. oryzae BECh-2 is described in Danish patent application PA 1999 01726. It is a mutant of JaL 228 (described in WO 98/12300) which is a mutant of IFO 4177.

Reagents

Media and reagents

Cove: 342.3 g/L Sucrose, 20 ml/L COVE salt solution, 10mM Acetamide, 30
5 g/L noble agar.

Cove-2: 30 g/L Sucrose, 20 ml/L COVE salt solution, 10mM, Acetamide, 30 g/L noble agar.

Cove salt solution: per liter 26 g KCl, 26 g MgSO4-7aq, 76 g KH2PO4, 50ml Cove trace metals.

10 Cove trace metals: per liter 0.04 g NaB4O7-10aq, 0.4 g CuSO4-5aq, 1.2 g FeSO4-7aq, 0.7 g MnSO4-aq, 0.7 g Na2MoO2-2aq, 0.7 g ZnSO4-7aq.

AMG trace metals: per liter 14.3 g ZnSO4-7aq, 2.5 g CuSO4-5aq, 0.5 g NiCl2, 13.8 g FeSO4, 8.5 g MnSO4, 3.0 g citric acid.

YPG: 4 g/L Yeast extract, 1 g/L KH2PO4, 0.5 g/L MgSO4-7aq, 5 g/L Glu-
15 cose, pH 6.0.

STC: 0.8 M Sorbitol, 25 mM Tris pH 8, 25 mM CaCl2.

STPC: 40 % PEG4000 in STC buffer.

Cove top agarose: 342.3 g/L Sucrose, 20 ml/L COVE salt solution, 10mM Acetamide, 10 g/L low melt agarose.

20 MS-9: per liter 30 g soybean powder, 20 g glycerol, pH 6.0.

MDU-pH5: per liter 45 g maltose-1aq, 7 g yeast extract, 12 g KH2PO4, 1 g MgSO4-7aq, 2 g K2SO4, 0.5 ml AMG trace metal solution and 25 g 2-morpholinoethanesulfonic acid, pH 5.0.

EXAMPLES

25 **Example 1: Cloning and expression of lipase gene from *Fusarium culmorum***

Transformation in *Aspergillus* strain

Aspergillus oryzae strain BECh-2 was inoculated to 100 ml of YPG medium and incubated for16 hrs at 32°C at 120 rpm. Pellets were collected and washed with 0.6 M KCl, and resuspended 20 ml 0.6 M KCl containing a commercial β-glucanase
30 product (Glucanex, product of Novo Nordisk A/S) at the concentration of 30 µl/ml. Cultures were incubated at 32°C at 60 rpm until protoplasts formed, then washed with STC buffer twice. The protoplasts were counted with a hematometer and resus-

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pended in an 8:2:0.1 solution of STC:STPC:DMSO to a final concentration of 2.5x10e7 protoplasts/ml. About 3 μg of DNA was added to 100 μl of protoplasts solution, mixed gently and incubated on ice for 30 min. One ml of SPTC was added and incubated 30 min at 37°C. After the addition of 10 ml of 50°C Cove top agarose, the
5 reaction was poured onto Cove agar plate. Transformation plates were incubated at 32°C for 5 days.

PCR screening of lipase

A strain of *Fusarium culmorum* was used as a genomic DNA supplier.

PCR reactions on *Fusarium culmorum* genomic DNA was done with two fol-
10 lowing primer set: lip2 / lip21 designed based upon the alignment 3 lipases from *Fusarium*.

lip2: 5'-aarttytayathcarcaygg-3'

lip21: 5'-tciswigtytgickrtaiccraa-3'

15 Reaction components (6 ng /μl of genomic DNA, 250 mM dNTP each, primer 250 nM each, 0.1 U/ μl in Taq polymerase in 1X buffer (Roche Diagnostics, Japan)) were mixed and submitted for PCR under the following conditions.

Step	Temperature	Time
1	94°C	1 min
2	50°C	1 min
3	72°C	2 min
4	72°C	10 min
5	4°C	forever

Steps 1 to 3 were repeated 30 times.

20 0.7 kbp of fragment was amplified. It was gel-purified with GFX™ PCR DNA and Gel Band Purification kit (amersham pharmacia biotech) and ligated into a pT7Blue vector with ligation high (TOYOBO, Japan). The ligation mixtures were transformed into *E. coli* JM109. The resultant plasmids, pT12-0221 was sequenced and compared to the *Fusarium oxysporum* lipase, showing that a clone encodes the internal part of
25 the lipase.

Cloning of lipase gene

In order to clone the missing part of the lipase gene, LA PCR™ in vitro Cloning Kit (TaKaRa) was used for genome walking. 0.5 kbp of DNA fragment corresponding to N-terimal region was obtained from BamH I digested genome ligated to

Sau3A I cassette of the kit with 12N1 primer. 1.8 kb of DNA fragment corresponding to C-terminal region was obtained from Bgl II digested genome ligated to Sau3A I cassette of the kit with 12C2 primer.

12N1: 5'-actgaacagttggacagccgtttccgctgc-3'
5 12C2: 5'-ccaggctggtggagagttccgcgttacgaacg-3'

Obtained fragments were purified by GFX™ PCR DNA and Gel Band Purification kit (amersham pharmacia biotech) and sequenced with each primer which amplified the fragment. Their sequence were compared to the Fusarium oxysporum 10 lipase, showing that the amplified DNA covered N-terminal and C-terminasl part of the lipase.

The fidelity of taq polymerase is not so good so in order to get the right sequence whole gene was amplified the following primers.

15 12-N (Bcl): 5'-ttgtctgtgatcatgcgtctcctgtcactcctc-3'
12-C(Sal): 5'-ttagtgcgtaaacagctgactatgatgagcggctggcgtgagtc-3'

Reaction components (6 ng /µl of genomic DNA, 250 mM dNTP each, primer 250 nM each, 0.05 U/ µl of Expand high fidelity polymerase in 1X buffer (Roche Di-20 agnostics, Japan)) were mixed and submitted for PCR under the following conditions.

Step	Temperature	Time
1	94°C	2 min
2	94°C	10sec
3	55°C	30sec
4	68°C	45sec
step 2-4 repeat 10 times		
5	94°C	10sec
6	55°C	30sec
7	68°C	45sec +20sec/cycle
step 5-7, repeat 20 times		
8	68°C	7min
7	4°C	forever

An amplified DNA fragment was gel-purified with GFX™ PCR DNA and Gel Band Purification kit (amersham pharmacia biotech) and ligated into a pT7Blue vector with ligation high (TOYOBO, Japan). The ligation mixtures were transformed into
5 *E. coli* JM109. The resultant plasmids, pT12-1, pT12-2, pT12-3, and pT12-4, were sequenced and all of them are identical. The sequence is defined as *Fusarium culmorum* lipase DNA sequence.

Expression of lipase gene in *Aspergillus oryzae.*

The plasmid pT12-6 was transformed to JM110 and non-methylated pT12-6
10 was extracted. The lipase gene was digested from non-methylated pT12-6 with Bcl I and Sal I into T-vector and ligated into the BamH I and XhoI sites in the *Aspergillus* expression cassette pMT2188 which has *Aspergillus niger* neutral amylase promoter, *Aspergillus nidulans* TPI leader sequences, *Aspergillus niger* glucoamylase terminator and *Aspergillus nidulans* amdS gene as a marker and Saccharomyces cerevisiae
15 URA3 gene as a marker for a plasmid construction. The ligation mixture was transformed E.coli 6507 by electroporation and the resultant plasmid was pNL12-***.

pNL12-*** was transformed into *Aspergillus oryzae* BECh-2. The selected transformants were inoculated in 100 ml of MS-9 media and cultivated at 30°C for 1 day. 3 ml of grown cell in MS-9 medium was inoculated to 100 ml of MDU-2BP me-
20 dium and cultivated at 32°C for 3 days. The supernatant was obtained by centrifugation.

The lipase productivity of selected transformants was determined as LU activity.

SEQUENCE LISTING

25 12N1: 5'-actgaacagttggacagccgtttccgctgc-3'

12C2: 5'-ccaggctggtggagagttccgcgttacgaacg-3'

12-N (Bcl): 5'-ttgtctgtgatcatgcgtctcctgtcactcctc-3'

12-C(Sal): 5'-ttagtgcgtaaacagctgactatgatgagcggctggcgtgagtc-3'

CLAIMS

1. A nucleic acid sequence which comprises:
   a) the partial DNA sequence encoding a mature lipolytic enzyme cloned into a plasmid present in *Escherichia coli* DSM 13537,
5 b) the partial DNA sequence encoding a mature lipolytic enzyme shown in SEQ ID NO: 1,
   c) an analogue of the sequence defined in a) or b) which encodes a lipolytic enzyme and
      i) has at least 80 % homology with said DNA sequence, or
10   ii) hybridizes at high stringency with a complementary strand of said DNA sequence or a subsequence thereof having at least 100 nucleotides,
      iii) is an allelic variant thereof, or
   d) a complementary strand of a), b) or c).

15 2. A nucleic acid construct comprising the nucleic acid sequence of claim 3 or 4 operably linked to one or more control sequences capable of directing the expression of the lipolytic enzyme in a suitable expression host.

3. A recombinant expression vector comprising the nucleic acid construct of claim 5, a promoter, and transcriptional and translational stop signals.

20 4. A recombinant host cell comprising the nucleic acid construct of claim 6.

5. A method for producing a lipolytic enzyme comprising cultivating the host cell of claim 7 under conditions conducive to production of the lipolytic enzyme, and recovering the lipolytic enzyme.

6. A method for preparing a dough or a baked product made from the dough, 25 comprising adding the lipolytic enzyme of claim 1 to the dough.

7. A dough composition comprising the lipolytic enzyme of claim 1.

8. A detergent composition comprising a surfactant and the lipolytic enzyme of claim 1.

SEQUENCE LISTING

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<130> Fusarium culmorum lipase gene (FL-12)

<140>
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<160> 2

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-30             -25                 -20             -15

cct ctg agc gtt gaa gaa tac gcc aag gct ctc gat gaa cga g      91
Pro Leu Ser Val Glu Glu Tyr Ala Lys Ala Leu Asp Glu Arg
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Ile Val Ala Ser Phe Thr
                55

aactccaagc ttacacgttt ctctcag t gga tcc aag act gga atc ggc ggc  391
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                85                  90                  95

gac gac tgc agc ctg acc tcg ggc tgt ggt gtt cac tca ggc ttc cag  535
Asp Asp Cys Ser Leu Thr Ser Gly Cys Gly Val His Ser Gly Phe Gln
            100                 105                 110

aaa gcc tgg aac gag atc tca gcc gcg gca acc gcc gct gtc gca aag  583
Lys Ala Trp Asn Glu Ile Ser Ala Ala Ala Thr Ala Ala Val Ala Lys
        115                 120                 125

gcc cgc aag gca aac cct tcg ttc aag gtc gtc tcc gta ggt cac tcc  631
Ala Arg Lys Ala Asn Pro Ser Phe Lys Val Val Ser Val Gly His Ser
    130                 135                 140

ctg ggt ggt gct gta gct aca ctg gca ggc gcg aac cta cga gtt ggt  679
Leu Gly Gly Ala Val Ala Thr Leu Ala Gly Ala Asn Leu Arg Val Gly
145                 150                 155                 160

gga aca ccc ctt gac att tac acc tac ggt tca ccc cga gtt gga aac  727
Gly Thr Pro Leu Asp Ile Tyr Thr Tyr Gly Ser Pro Arg Val Gly Asn
                165                 170                 175

aca cag ctc gct gct ttt gtc tcg aac cag gct ggt gga gag ttc cgc  775
Thr Gln Leu Ala Ala Phe Val Ser Asn Gln Ala Gly Gly Glu Phe Arg
            180                 185                 190

gtt acg aac gcc aaa gac ccc gtg cct cgt ctc ccc cct ctg atc ttt  823
Val Thr Asn Ala Lys Asp Pro Val Pro Arg Leu Pro Pro Leu Ile Phe
        195                 200                 205

gga tac cga cac aca tcc ccc gag tac tgg ctg tct ggc agc gga ggt  871
Gly Tyr Arg His Thr Ser Pro Glu Tyr Trp Leu Ser Gly Ser Gly Gly
    210                 215                 220

gac aag atc gac tac acc atc aac gat gtc aag gtc tgt gaa ggt gcc  919
Asp Lys Ile Asp Tyr Thr Ile Asn Asp Val Lys Val Cys Glu Gly Ala
225                 230                 235                 240

gcc aac ctc cag tgc aac ggt gga aca ctc gga ttg gat atc gat gcc  967
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Ala Asn Leu Gln Cys Asn Gly Gly Thr Leu Gly Leu Asp Ile Asp Ala
245 250 255

cat ctc cac tac ttc cag gca act gat gct tgc tct gct ggc ggc atc 1015
His Leu His Tyr Phe Gln Ala Thr Asp Ala Cys Ser Ala Gly Gly Ile
260 265 270

tcg tgg aga aga tac agg agc gcc aag cgt gag agc atc tca gag agg 1063
Ser Trp Arg Arg Tyr Arg Ser Ala Lys Arg Glu Ser Ile Ser Glu Arg
275 280 285

gct acc atg acc gac gcc gag ctc gag aag aag ctc aac agc tat gtt 1111
Ala Thr Met Thr Asp Ala Glu Leu Glu Lys Lys Leu Asn Ser Tyr Val
290 295 300

gag atg gat aag gag tat atc aag act cac gcc agc cgc tca tca tag 1159
Glu Met Asp Lys Glu Tyr Ile Lys Thr His Ala Ser Arg Ser Ser
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<213> Fusarium sp.

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Pro Leu Ser Val Glu Glu Tyr Ala Lys Ala Leu Asp Glu Arg Ala Val
20 25 30
Ser Val Ser Thr Thr Asp Phe Gly Asn Phe Lys Phe Tyr Ile Gln His
35 40 45
Gly Ala Ala Ala Tyr Cys Asn Ser Glu Ala Pro Ala Gly Ala Lys Val
50 55 60
Thr Cys Ser Gly Asn Gly Cys Pro Thr Val Gln Ser Asn Gly Val Thr
65 70 75 80
Ile Val Ala Ser Phe Thr Gly Ser Lys Thr Gly Ile Gly Gly Tyr Val
85 90 95
Ala Thr Asp Pro Thr Arg Lys Glu Ile Val Val Ser Phe Arg Gly Ser
100 105 110
Ile Asn Ile Arg Asn Trp Leu Thr Asn Leu Asp Phe Asp Gln Asp Asp
115 120 125
Cys Ser Leu Thr Ser Gly Cys Gly Val His Ser Gly Phe Gln Lys Ala
130 135 140
Trp Asn Glu Ile Ser Ala Ala Ala Thr Ala Ala Val Ala Lys Ala Arg
145 150 155 160
Lys Ala Asn Pro Ser Phe Lys Val Val Ser Val Gly His Ser Leu Gly
165 170 175
Gly Ala Val Ala Thr Leu Ala Gly Ala Asn Leu Arg Val Gly Gly Thr
180 185 190
Pro Leu Asp Ile Tyr Thr Tyr Gly Ser Pro Arg Val Gly Asn Thr Gln
195 200 205
Leu Ala Ala Phe Val Ser Asn Gln Ala Gly Gly Glu Phe Arg Val Thr
210 215 220
Asn Ala Lys Asp Pro Val Pro Arg Leu Pro Pro Leu Ile Phe Gly Tyr
225 230 235 240
Arg His Thr Ser Pro Glu Tyr Trp Leu Ser Gly Ser Gly Gly Asp Lys
245 250 255
Ile Asp Tyr Thr Ile Asn Asp Val Lys Val Cys Glu Gly Ala Ala Asn
260 265 270
Leu Gln Cys Asn Gly Gly Thr Leu Gly Leu Asp Ile Asp Ala His Leu

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    275                 280                 285
His Tyr Phe Gln Ala Thr Asp Ala Cys Ser Ala Gly Gly Ile Ser Trp
   290                 295                 300
Arg Arg Tyr Arg Ser Ala Lys Arg Glu Ser Ile Ser Glu Arg Ala Thr
305                 310                 315                 320
Met Thr Asp Ala Glu Leu Glu Lys Lys Leu Asn Ser Tyr Val Glu Met
                325                 330                 335
Asp Lys Glu Tyr Ile Lys Thr His Ala Ser Arg Ser Ser
            340                 345
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